THE DEMONSTRATION OF SARCOPLASMIC RETICULUM IN BOVINE MUSCLE

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Plates 42–44

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SYNOPSIS

The fine sarcoplasmic network described by a number of workers, and demonstrated by Veratti using a Golgi-type reaction, has been studied in several bovine muscles. The similarity in the appearance of this network in the muscles examined is noted, and suggestions made to explain the slight variations encountered. Various features of the reticular pattern are discussed with reference to myofibrillar arrangement within the muscle fibre.

Introduction

Recent work by a number of electron microscopists (Bennett & Porter, 1953; Ruska, 1954; Weinstein, 1954; Porter *et al.*, 1956) has directed attention to the fine structure of muscle tissue. In particular, the sarcoplasmic component of such structure has been the subject of intensive investigation (Porter *et al.*, 1961). Interest in the fine network, currently referred to as "sarcoplasmic reticulum", which is to be found in muscle fibres, dates back to the turn of the century. Indeed, as Smith (1961) points out in a useful historical review of light-microscope studies in muscle structure, a number of early workers (Thin, 1876; Retzius, 1881; Cajal, 1888, 1890) described networks of one kind or another. Some of these descriptions have been aligned with more recent findings.

The classic work of Veratti (1902, 1961) in which he describes the fine network observed in muscle tissue following the black reaction of Golgi has proved to be of remarkable significance in the identification of the sarcoplasmic reticulum. Excellent drawings by Veratti show the pattern of this network in a number of species representing mammals, birds, reptiles, amphibia, fish, insects, and crustaceans. Studies on mice and insects using the electron microscope have led recent workers (as indicated by Bennett, 1960), to conclude that the sarcotubular system they have observed must be very similar or even identical to the fine network described by Veratti.

Some experiments involving histological study of certain bovine muscles (Penny, Voyle & Lawrie, 1963) led us to consider the sarcoplasmic reticulum in fresh tissue. Veratti's procedure was adopted as being convenient for light-microscope studies, bearing in mind the limitations of the method. The pattern of the fine network observed in fibres of these muscles was compared and photomicrographs prepared.

Material and Methods

Tissue was obtained under conditions described elsewhere (Penny, Voyle & Lawrie, 1963) from post-rigor muscles of beef steers of Aberdeen Angus × Jersey breed. The muscles used were *biceps femoris*, *semimembranosus*, *longissimus dorsi*, *psoas major*, and *pectoralis profundus*. Pieces of tissue approximately 2 mm thick were trimmed to give longitudinal and transverse planes of section. The procedure adopted by Veratti (1902) was used with minor modifications; tissue blocks were treated according to the following schedule.

1. Osmic acid–potassium dichromate mixture	5 days
Wash in three changes of distilled water	
2. 1 p.c. aqueous silver nitrate (in the dark)	1 day
Wash in three changes of distilled water	
3. Osmic acid–potassium dichromate mixture	2 days
Wash in three changes of distilled water	
4. 1 p.c. aqueous silver nitrate (in the dark)	5 days
Wash in distilled water	

Tissue samples were then dehydrated by passing through increasing concentrations of alcohol, cleared in cedarwood oil or chloroform and embedded in paraffin wax. Sections were cut at 5 μ and mounted on slides; after drying, the wax was removed from the section with xylol and a cover-slip added using dammar xylol as mountant.

The osmic acid–potassium dichromate mixture was prepared as follows:

Osmic acid 1 p.c. aq.	1 part
Potassium dichromate 5 p.c. aq.	4 parts

Fresh tissue or material which had been held at $-20°C$ gave satisfactory results with the above procedure, though it was found that the method would not work on material which had been initially fixed in formalin. As is often found with Golgi-type preparations, mounted sections could not be regarded as permanent. Such sections faded in two to three months and were then unusable. Sectioned material will retain the impregnation indefinitely provided it is not dewaxed and mounted until required for examination.

Results

Veratti (1902) reported, and the present results confirm, that the end-product of the method described above is an incompletely impregnated piece of tissue. Some fibres became impregnated in whole or in part as seen in transverse section, others show no reaction at all. The appearance of a longitudinal section of fibres shows that impregnation occurs in a sporadic manner. Sections of *semimembranosus* in which incomplete impregnation was found are shown in Pl. 42, fig. 1, 2.

The pattern of the sarcoplasmic reticulum in the different muscles examined showed little variation. In longitudinal sections of all five muscles a regular arrangement of transverse strands or reticula was observed. At its best this arrangement extended over the full width of the fibre, though the sarcolemma did not appear to be impregnated. Nuclei too remained unaffected. Sections examined under phase-contrast illumination suggested that the parallel transverse strands were situated on either side of the Z-line, presumably at the A-I band junction.

Longitudinal strands were also observed in all the muscles examined. These fine strands showed some degree of register in forming connections between successive pairs of

transverse reticula. The strands were irregularly spaced across the fibre and were not apparent in all areas. There were, however, more to be seen in sections of *psoas* and *pectoralis profundus* than in *semimembranosus* and *biceps femoris*. *Longissimus dorsi* showed moderate numbers of connecting strands. This variability in number and disposition will be considered later. Pl. 43, fig. 3–7 shows the arrangement of the sarcoplasmic network in longitudinal sections of the muscles examined.

Another notable feature in longitudinal sections was the regularly spaced thickenings or nodes in the transverse strands. These nodes occurred in register both laterally and transversely. In addition heavily impregnated areas scattered throughout the sarcoplasm were found in both longitudinal and transverse sections. In an editor's note included in the translation of Veratti's paper (Veratti, 1961), it is suggested that these areas represent mitochondria or sarcosomes.

In transverse section an open network pattern was observed in all muscles studied. The configuration of the pattern varied slightly from fibre to fibre but generally speaking the network enclosed spaces of fairly uniform size and shape. This appearance suggests that the reticulum surrounds individual myofibrils, a suggestion which is confirmed by electron-microscope observations (Porter & Palade, 1957). Points of attachment of the reticulum to the sarcolemma were observed in some instances. Pl. 44, fig. 8 represents a transverse section of *semimembranosus*, which is typical of the muscles studied.

Discussion

The incomplete reaction which is a feature of the impregnation method described above (and, indeed, of any Golgi-type method) may be regarded as limiting the usefulness of the technique. Pl. 42, fig. 1, 2, show the random nature of the reaction between fibres as well as within individual fibres. As Veratti pointed out, it cannot therefore be concluded that absence of reaction indicates an absence of the sarcoplasmic reticulum. In examining tissue samples by this method after accelerated freeze-drying and subsequent reconstitution it was consistently found that no impregnation occurred. Electron micrographs, however, show that sarcoplasmic reticulum is still present.

Veratti also drew attention to the variable reaction obtained, in respect of the location of strands, in different fibres of the same muscle as well as variation occasionally found in the same fibre. The number of transverse strands varied between one and three in each "muscle unit" or sarcomere. His conclusion was that only one type of structure existed, i.e. one composed of three transverse strands and that the variable picture was due to incomplete metallic impregnation.

In all the muscles which we have studied we have consistently found fibrils to be of the "double-reticulum" type, i.e. twin parallel strands of impregnation, such as are shown in Pl. 43, fig. 3–7 and in Pl. 44, fig. 9.

In comparing light and electron microscopy of this component of muscle structure the thickness of section used in each case must be taken into account. This was of the order of 5 μ in paraffin sections and accordingly the transverse reticula seen in longitudinal sections were apparently continuous. However, ultra-thin sections of 50 mμ thickness—$\frac{1}{100}$th that of the paraffin sections—show the reticulum as a discontinuous structure at the given level of section. A three-dimensional picture serves to demonstrate the continuous nature of this system as is shown by Bennett (1956).

The deviations from a parallel arrangement of this reticulum, which may be seen in an enlarged portion of the photomicrograph of *longissimus dorsi* (L.S.) (Pl. 44, fig. 9), may be explained on the basis of the arrangement of myofibrils within a fibre. Similarly, the

irregular spacing of longitudinal strands, where these have been successfully impregnated, is due to the disposition of the myofibrils. These strands, some of which show a notable degree of alignment (Pl. 44, fig. 9), indicate the continuity of the network throughout the fibre. This impression is confirmed by observing transverse and longitudinal sections concurrently. Porter and Palade (1957) have produced several drawings showing a three-dimensional aspect of the sarcoplasmic reticulum in different muscles they have examined.

The regularity of the nodes or thickenings observed on the transverse strands in longitudinal section suggests that these are associated with some specific feature of the sarcoplasmic network. Since the reticulum surrounds each myofibril it may be assumed that a section saggital to the plane of Pl. 44, fig. 9, would have a similar appearance. Therefore the reticulum running approximately perpendicular to that seen in this photograph will intersect with and, it would appear from some areas, anastomose with the remainder of the network. It is at these intersections that the thickenings appear and the inequality in their lateral spacing may well be a result of the disposition of the myofibrils. Such thickenings do not appear so frequently in transverse section, and in some instances may be obscured by much heavier impregnation of sarcosomes lying in interfibrillar spaces.

Further investigations of the sarcoplasmic network in these muscles are being carried out using electron microscopy.

Acknowledgments

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DESCRIPTION OF PLATES 42–44 (after p. 178)

PLATE 42

FIG. 1.—Low power micrograph of *semimembranosus* in longitudinal section, showing partial impregnation of tissue. Dark areas represent impregnated sarcoplasmic reticulum.

Fig. 2.—*Semimembranosus* in transverse section showing partial impregnation as in Fig. 1.

PLATE 43

Fig. 3.—High-power micrograph of *biceps femoris* (L.S.) showing transverse pattern of sarcoplasmic reticulum.

Fig. 4.—Section of *longissimus dorsi* (L.S.) showing similar pattern of sarcoplasmic reticulum. A number of longitudinal reticula may be seen at the right of the micrograph.

Fig. 5.—Section of *semimembranosus* (L.S.). Several areas show anastamosing reticula.

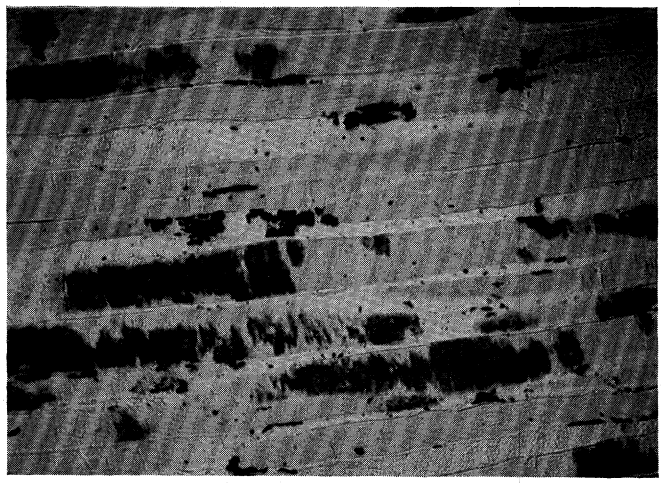
Fig. 6.—Section of *psoas major* (L.S.). Numerous longitudinal strands may be seen joining successive pairs of transverse reticula.

Fig. 7.—Section of *pectoralis profundus* (L.S.). This also shows numerous longitudinal reticula.

PLATE 44

Fig. 8.—Transverse section of single fibre of *semimembranosus* (enlarged from high power micrograph) showing network of sarcoplasmic reticulum. Nodes and sarcosomes are indicated. Part of the fibre which failed to impregnate is shown on the right of the micrograph at "X".

Fig. 9.—Enlargement of part of fig. 4, showing longitudinal strands (lr) connecting successive pairs of transverse reticula, nodes at intersections of the reticulum, and sarcosomes.



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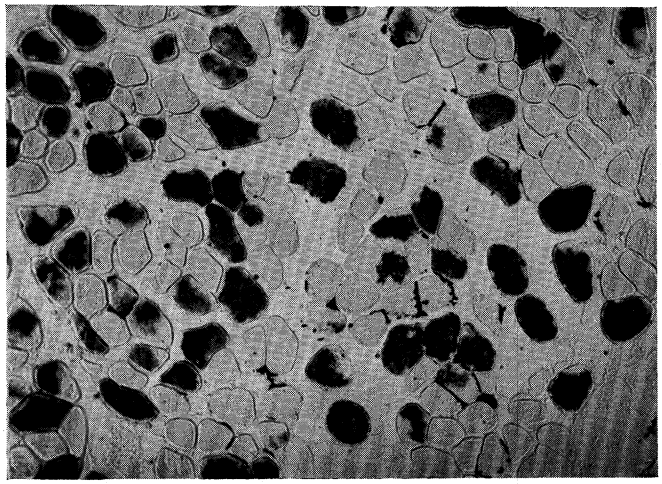
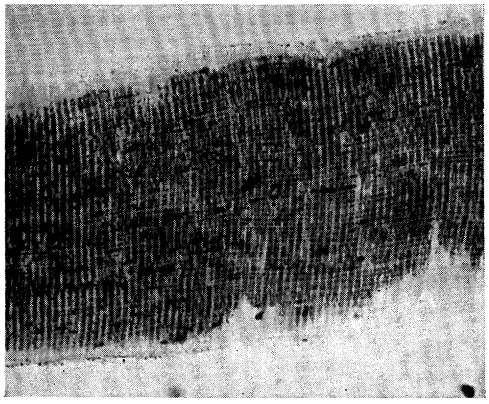


2

100μ



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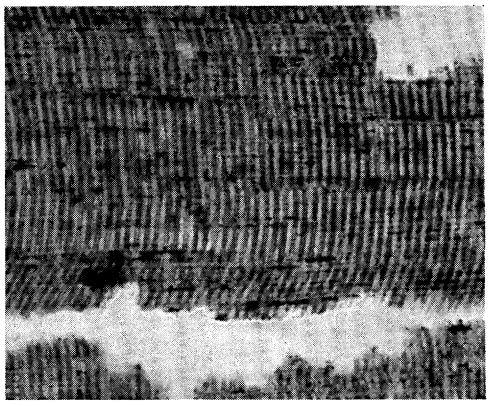

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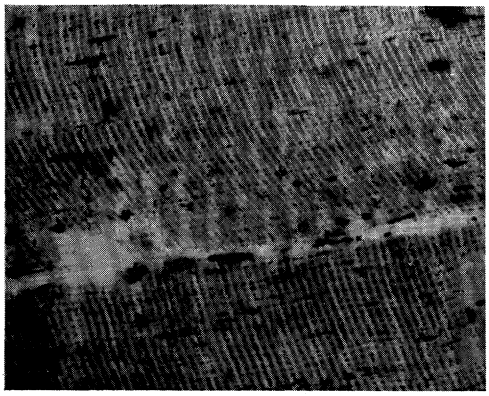

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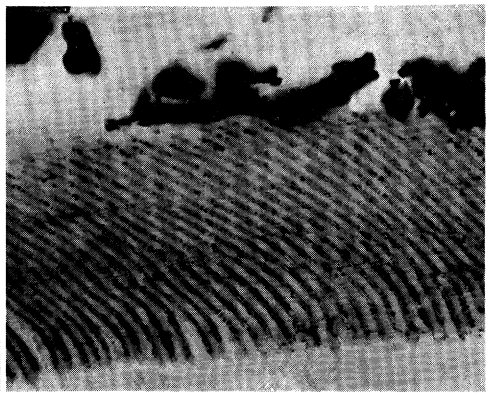

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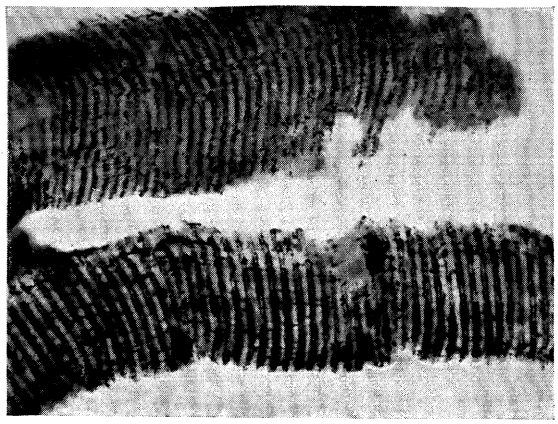
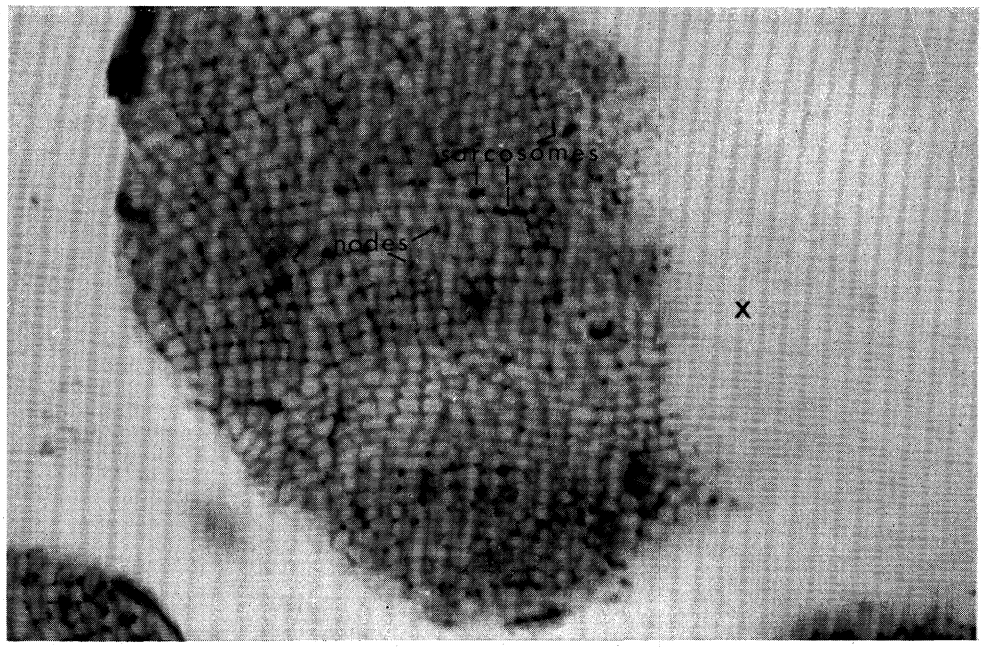
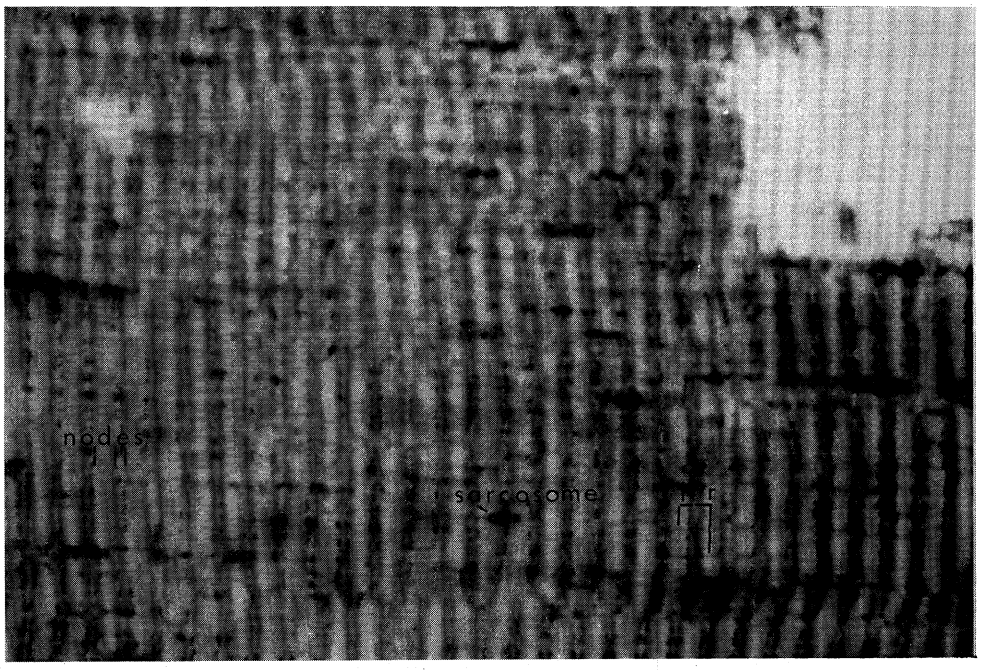


7

10M





8(above)
9(below)

10 μ